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Microbial Basis for Enhanced Degradation of the Fumigant 1,3-Dichloropropene (1,3-D) in Soil

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ABSTRACT

The differential enhanced degradation of cis- and trans-1,3-D was observed in the previous two studies performed by Ou et al. (1995) and especially Chung et al. (1999). This study was initiated to investigate the involvement of microorganisms in the differential enhanced degradation of the chemicals. As expected, microorganisms were responsible for the enhanced degradation of the chemicals. A mixed bacterial culture capable of degrading 1,3-D was isolated from an enhanced soil sample collected from a site treated with 1,3-D. Similar to the enhanced soil, the mixed culture degraded trans-1,3-D faster than cis-1,3-D. This mixed culture could not utilize cis- and trans-1,3-D as a sole source of carbon for growth. Rather, a variety of second substrates were evaluated to stimulate the differential enhanced degradation of the two isomers. As a result, the mixed culture degraded cis- and trans-1,3-D only in the presence of a suitable second substrate. Second substrates that had the capacity to stimulate the degradation included soil leachate, tryptone, tryptophan, and alanine. Other substrates tested, including soil extract, glucose, yeast extract, and indole failed to stimulate the degradation of the two isomers. Therefore, it appeared that the degradation of cis- and trans-1,3-D was a cometabolic process. The mixed culture was composed of four morphologically distinctive bacterial colonies.

Key words: Cis- and trans-1,3-D, cometabolism, bacterial consortium, second carbon substrate (source)

INTRODUCTION

The gaseous fumigant methyl bromide will be banned by USEPA in 2001 due to its ozone depletion (Noling and Becker, 1994). The liquid fumigant 1,3-dichloropropene (1,3-D) is considered to be a potential alternative to methyl bromide (Anonymous, 1995). 1,3-dichloropropene consists of the two isomers, cis- and trans-1,3-D. Chemical and physical properties of cis- and trans-1,3-D were shown in Table 1 (DowElanco, 1996).

It is well known that the field soil contaminated with toxic organic chemicals have shown rapid degradation of the chemicals, because microorganisms have gradually been adapted to the chemicals (Atlas and Bartha, 1986). Microorganisms appear to be responsible for enhanced degradation of pesticides which are toxic organic chemicals (Racke and Coats, 1990). However, similar to other short-chain halogenated hydrocarbons, microorganisms capable of utilizing 1,3-D as a sole source of carbon for growth and energy have not been isolated from Florida sandy soils.

Two studies have reported successful isolation of axenic bacterial cultures capable of degrading 1,3-D from Dutch soils (Lebbink et al., 1989; Verhagen et al., 1995). Lebbink et al. (1989) isolated a strain of a *Pseudomonas* sp. from a soil that had been continuously treated with 1,3-D for 12 years. They claimed that the isolate was able to utilize 1,3-D as a sole source of carbon for its energy and growth. However, it is questionable that this isolate indeed could utilize 1,3-D as a sole source of carbon for growth. The isolate was maintained in a rich liquid medium containing several biodegradable organic chemicals. The *Pseudomonas* sp. degraded trans-1,3-D more rapidly than cis-1,3-D. This supported the findings of Ou et al. (1995) and Chung et al. (1999) that trans-1,3-D in enhanced soils was degraded more rapidly than cis-1,3-D. Verhagen et al. (1995) isolated six axenic bacterial cultures capable of utilizing cis-1,3-D as a sole source of carbon and energy from soil samples collected from microplots that had been intensively treated with cis-1,3-D for a span of one year. Again, it was questionable that these isolates were truly capable of solely utilizing cis-1,3-D for growth, because yeast extract was used for maintaining these cultures. The isolates harbored plasmids and Verhagen et al. (1995) suggested that the plasmids were responsible for the degradation of cis-1,3-D. It is not known whether these isolates also had the capacity to degrade

trans-1,3-D.

Roberts and Stoydin (1976) demonstrated that cis- and trans-1,3-D was hydrolyzed to their corresponding 3-chloroallyl alcohols (3-CAA), which were then oxidized to 3-chloroacrylic acids (CCAs) in soil, respectively (Figure 1). 3-Chloroallyl alcohol which is the key metabolite of 1,3-D was also found to be rapidly degraded in soil (Leistra et al., 1991; Van Dijk, 1980). A strain of *Pseudomonas* sp. isolated from a California soil had the capacity to utilize 3-CAA as a sole source of carbon for growth (Belser and Castro, 1971). Van Waarde et al. (1993) also isolated three strains of *Pseudomonas* sp. from a Dutch soil capable of utilizing 2-chloroallyl alcohol, an analogous chemical of 3-chloroallyl alcohol, as a sole source of carbon for growth. Thus, it appears that chloroallyl alcohols can serve as a sole source of carbon for a number of soil microorganisms, especially *Pseudomonas* sp.

The objective of this study was to isolate microorganisms capable of degrading cis- and trans-1,3-D and screen for a second carbon substrates to stimulate the degradation of those chemicals.

MATERIALS AND METHODS

Chemicals

Telone II (94% commercial grade), analytical grade cis- and trans-1,3-D (98-99% purity) were provided by DowElanco Corporation (Indianapolis, IN). All other chemicals were pesticide-grade, analytical grade, or the highest grade commercially available.

Culture Media

Two basic media, basal mineral-trace mineral media (BMTMM) and soil extract, were used. Other culture media used in this study were modified from the two basic media. Two types of soil extracts were used as well. They were soil extract (Ou, 1991) and soil leachate. The basal mineral medium was composed of K_2HPO_4, 4.8 g; KH_2PO_4, 1.2 g; NH_4NO_3, 1 g; $MgSO_4$ $7H_2O$, 0.25 g; $CaCl_2$, 0.04 g; $Fe_2(SO_4)_3$ $7H_2O$, 0.001 g; and deionized H_2O, 1 L. The pH of the medium was adjusted to 7.2. Stock trace minerals were made up of $MnSO_4$ $6H_2O$, 2 mg; $CuSO_4$ $5H_2O$, 1 mg; $ZnSO_4$ $6H_2O$, 2 mg; H_3BO_3, 0.3 mg; Na_2MoO_4 $2H_2O$, 0.4 mg;

$CoCl_2$ $2H_2O$, 0.4 mg; and deionized H_2O, 1 L (Ou and Thomas, 1994). The stock trace minerals were sterilized by filtration. When supplemented, 500 L of the stock trace minerals was added to 50 mL of sterile MMA.

Preparation of Soil Extract

Soil extract was prepared by the procedure of Ou (1991). One kg of field-treated soil and 1 liter of deionized water were added to a 2 L Erlenmyer flask, and the flask was autoclaved at 121°C for 1 hour. After soil particles had settled overnight, the supernatant was carefully poured into 500 mL plastic centrifuge bottles, and the bottles were centrifuged at a speed of 10,000 rpm. Nalgene microfilters (<0.22 μm) were used for filtration and sterilization of the centrifuged soil extract, with the extract being kept in a refrigerator (4°C) until use.

Preparation of Soil Leachate

Five hundred grams of field-treated soil and 250 mL of deionized water (2 to 1 ratio) were added to a 1 L Erlenmyer flask and then shaken for 10 minutes. Soil particles were allowed to settle overnight, and the supernatant was transferred to 500 mL plastic centrifuge bottles. These bottles were then centrifuged at a speed of 5,000 rpm for 20 minutes. Nalgene microfilters (<0.22 μm) were used for filtration and sterilization of the extract to keep heat- labile soil nutrients intact. The filtered extract was maintained at room temperature (25°C) until use.

Preparation of L-Plates and L-Broth

L-agar plates - which are composed of 200 mL of deionized water, 2 g of tryptone, 1 g of yeast extract, 1 g of NaCl, and 4 g of agar - were used for isolation of axenic cultures from mixed cultures and for checking purity of axenic cultures. L-broth was the same as for the L-plates, except that agar was not added.

Preparation of Yeast Extract, Tryptone, Tryptophan, Indole, Alanine Solutions

One percent solutions of yeast extract, tryptone, tryptophan, indole and alanine in deionized water were prepared, and were sterilized by autoclaving.

Enrichment and Isolation of Cis- and Trans-1,3-D Degrading Microorganisms

The basal mineral-trace mineral medium (BMTMM), when containing a small amount of enhanced soil (0.01 g/mL), was found to have the capacity to degrade cis- and trans-1,3-D. Small amounts of this suspension then were streaked on L-agar plates. Individual colonies did not have the capacity to degrade cis- and trans-1,3-D when grown in BMTMM supplemented with soil leachate (0.04 mL/mL), but a mixed bacterial culture from a mixed bacterial colony grown in the same medium was found to have the capacity to degrade the two isomers. Table 2 shows various culture media containing various biodegradable second carbon sources that were used for screening a second carbon substrate capable of stimulating 1,3-D degradation.

Fifty mL of each test medium (Table 2) was placed in a 250 mL glass flask with a Teflon-lined cap. To prevent leaks, the top of each flask was wrapped with Teflon tape. After cis- and trans-1,3-D (25 μg/mL each) were added, the flask was immediately closed with the cap. To prevent photodegradation, all flasks were wrapped with aluminum foil, and incubated on a rotary shaker at 28°C. At desired time intervals, 0.5 mL of the culture fluids was removed and transferred to a glass culture tube for hexane extraction.

Culture Extraction

Ten mL of cold hexane were added to a culture tube containing 0.5 mL of a culture fluid, and the tube was shaken on a reciprocal shaker at 500 strokes per minute for 10 minutes. After shaking, one mL of the hexane extract was transferred to a GC vial for GC analysis of cis- and trans-1,3-D.

GC Analysis

The analytical procedures for quantification of cis- and trans-1,3-D, and cis- and trans-3-CAA, were similar to the procedures previously described by Ou et al. (1995). Cis- and trans-1,3-dichloropropene and their corresponding hydrolysis products, cis- and trans-3-CAA, were quantified by a Perkin Elmer Autosystem GC equipped with an autosampler, ^{63}Ni electron capture detector (ECD), split-splitless injector, Turbochrom 4 software, and a 486 computer. The GC parameters and operational conditions were as follows:

column, 30 m x 0.25 mm i.d., RTX-624 coated with 3 μm film thickness; flow rates for carrier gas (He) and make-up gas (99.5% N_2 and 0.5% CH_4), 5 mL/minute and 30 mL/minute, respectively; injector temperature, 150°C; detector temperature, 375°C; oven temperature, 50°C for the first minute, ramp at 40°C/minute, and hold at 120°C for 16 minutes; split valve, off for the first 1 minute for analysis of 1,3-D or the first 1.5 minutes for analysis of 3-CAA; and injection volume of 1 $\mu\ell$ for 1,3-D and 3-CAA. Under these conditions, the retention times for cis- and trans-1,3-D and cis- and trans-3-CAA were 4.7, 5.0, 5.4, and 5.6 minutes, respectively. Limits of detection (Hubaux and Vos, 1970 and Ott, 1977) for cis- and trans-1,3-D and cis- and trans-3-CAA were 0.26, 0.28, and 0.66, and 0.54 μg/mL, respectively.

RESULTS

The degradation of cis- and trans-1,3-D was more rapid in the treated (enhanced) soil than the untreated (nonenhanced or control) soil and autoclaved soil (Figure 2). Trans-1,3-D was degraded faster than cis-1,3-D in treated (enhanced soil), whereas, the cis- and trans-1,3-D were degraded at a similar rate in both untreated (nonenhanced or conrol) and autoclaved soils. Those findings supported the hypothesis hat microorganisms were involved in the differential enhanced degradation of cis- and trans-1,3-D. Therefore, the experiments were performed to isolate microorganisms to degrade the cis- and trans-1,3-D. As mentioned in the Materials and Methods section, cis- and trans-1,3-D were also degraded in basal mineral-trace mineral medium (BMTMM) containing a small amount of nonsterile enhanced soil, but not in the same medium containing autoclaved enhanced soil (Table 3). Even though 1,3-D was degraded in BMTMM supplemented with a small amount of nonsterile enhanced soil, no visible turbidity was developed in the medium. Development of turbidity is a sign of bacterial growth. Furthermore, inoculation of nonsterile enhanced soil to BMTMM amended with autoclaved enhanced soil resulted in degradation of 1,3-D. It was obvious that the enhanced soil not only contained 1,3-D degrading bacteria but also provided an essential second carbon substrate that was capable of stimulating 1,3-D degradation. Without the second carbon substrate, the 1,3-D degrading bacteria could not degrade the chemical. As also mentioned in

the Materials and Methods section, a mixed bacterial culture was isolated, and the mixed culture had the capacity to degrade 1,3-D when grown in BMTMM supplemented with a small amount of soil leachate sterilized by filtration (Table 4). Even though the mixed culture grew well in soil extract, degradation of 1,3-D was not observed. Therefore, it appeared that the second carbon substrate that had the capacity to stimulate 1,3-D degradation was a heat-labile organic compound. It was costly and very difficult to obtain sufficient volume of soil leachate and, in addition, the mixed bacterial culture grew poorly and degraded 1,3-D slowly in BMTMM supplemented with soil leachate. By accident, it was observed that the mixed bacterial culture in BMTMM supplemented with a small amount of L-broth (10 $\mu\ell$/mL) rapidly degraded cis- and trans-1,3-D (Table 4), and also grew well in this medium. Although growth of the mixed culture in the full-strength L-broth was excellent, degradation of 1,3-D was not observed. L-broth consists of two organic components, yeast extract and tryptone. Yeast extract and tryptone (in BMTMM) thus were individually tested for their capacity to stimulate 1,3-D degradation by the mixed culture (Table 4). Only tryptone had the capacity to stimulate 1,3-D degradation. Tryptophan is the dominant amino acid of tryptone (Difco Laboratories, 1953). Tryptophan in BMTMM was then found to have excellent capacity to stimulate 1,3-D degradation by the mixed culture, even better than tryptone. Furthermore, alanine stimulated 1,3-D degradation by the mixed culture (Table 4). The mixed culture failed to degrade 1,3-D when grown in BMTMM supplemented with a readily degradable simple sugar, glucose, however.

1,3-Dichloropropene is subject to rapid chemical hydrolysis in aqueous media (McCall, 1987). The hydrolysis rate depends on temperature but is independent of pH at each temperature. Therefore, controls were always set up and incubated along with test culture media at the same time on the same shaker at 28°C. For those second carbon substrates capable of stimulating 1,3-D degradation, 1,3-D was completely degraded in 2 to 4 days by the mixed culture whereas, after 4 days, 60% of the 1,3-D remained in the controls. Trans-1,3-D was degraded faster than cis-1,3-D by the mixed culture if a suitable second carbon substrate was present, whereas the two isomers in the controls were degraded at the same rate. The mixed culture consisted of four morphologically distinctive bacterial colonies, as distinguished from their respective morphology.

DISCUSSION

Despite extensive efforts that have been made by Ou and his coworkers, microorganisms capable of utilizing cis- and trans-1,3-D as a sole source of carbon for growth could not be isolated from formerly treated soil. However, I did isolate a mixed bacterial culture capable of degrading cis- and trans-1,3-D from a treated soil. This culture degraded cis- and trans-1,3-D only in the presence of a second carbon substrate, such as tryptone or tryptophan. Therefore, the degradation appears to be a cometabolic process. Since tryptophan is the dominant amino acid of tryptone, it is understandable that either of these compounds can serve interchangeably as a second carbon substrate for the mixed bacterial culture which degrades cis- and trans-1,3-D. It is not clear why tryptophan is able to stimulate the mixed culture to produce the necessary enzymes for degradation of the two isomers, because 1,3-D and tryptophan are not structurally related. Tryptophan consists of an indole and a side chain (alanine), whereas 1,3-D is a short-chain chlorinated hydrocarbon, consisting of a three-carbon chain. However, tryptophan can be microbially degraded to indole and alanine (Mallette et al., 1979). The role of indole and/or alanine thus needed to be tested for their capacity to stimulate the degradation of 1,3-D. As a result, alanine stimulated 1,3-D degradation, but not indole. It was expected, since both 1,3-D and alanine consist of a three-carbon chain.

In the presence of soil leachate, the mixed bacterial culture had the capacity to degrade cis- and trans-1,3-D but not in the presence of soil extract. Thus, it is likely that the chemical(s) in the soil leachate that served as a second carbon substrate for the degradation of 1,3-D is/are heat- labile. Tryptophan is heat-labile, and is subject to acid hydrolysis, especially at high temperatures (Difco Laboratories, 1953). Since soil leachate may consist of a large number of organic chemicals, other chemical(s) may also serve as a second carbon substrate for the mixed culture to degrade 1,3-D as well.

Trans-1,3-D was degraded faster than cis-1,3-D both in enhanced soil and by the mixed culture. Thus, it is possible that the bacteria of the mixed culture might also be responsible for degradation of the two isomers in the soil. At present, it is not known whether two different enzymes are responsible for the hydrolysis, with one being specific for cis-1,3-D and the other for trans-1,3-D, and/or if the one specific for trans-1,3-D has higher

enzymatic activity than its counterpart. A single enzyme could also be responsible for the hydrolysis of both cis- and trans-1,3-D, but could have a higher activity toward trans-1,3-D, resulting in more rapid hydrolysis of the isomer.

I also entertained the idea that trans-1,3-D was isomerized to cis-1,3-D by microorganisms, resulting in more rapid degradation of the trans-1,3-D. However, I failed to validate this hypothesis. The (S)-fluazifop in soil was found to be enantiomerized to (R)-fluazifop (Bewick, 1986). Several soil bacteria have been found to have the capacity to utilize racemic 1,3-D or cis-1,3-D as a sole source of carbon for growth (Lebbink et al., 1989; Verhagen et al., 1995). However, these organisms were generally maintained in rich media. Thus, it is questionable that these bacteria could actually utilize 1,3-D or cis-1,3-D as a sole source of carbon for growth. These bacteria may instead cometabolize 1,3-D using some organic component of the medium as a second carbon substrate.

At present, little information is available about the genetics, molecular biology, and microbial ecology involved in the differential degradation of cis- and trans-1,3-D. Therefore, additional research toward understanding the biodegradation of cis- and trans-1,3-D, and their key degradation products cis- and trans-3-CAA and cis- and trans-3-chloroacrylic acid, is needed.

CONCLUSIONS

Microorganisms capable of utilizing 1,3-D as a sole source of carbon for growth could not be isolated from either enhanced or nonenhanced soils. A mixed bacterial culture that was isolated from an enhanced soil had the capacity to degrade 1,3-D. This mixed culture degraded 1,3-D in the presence of a second substrate such as tryptone, tryptophan, and alanine, but not in the presence of glucose, yeast extract or indole. The mixed culture degraded trans-1,3-D faster than cis-1,3-D. This mixed culture consisted of 4 morphologically distinctive bacterial colonies. It appeared that bacteria cometabolically and differentially degraded cis- and trans-1,3-D in enhanced soil.

REFERENCES

1. Annonymous, 1995. California says, "Hello, Telone." Ag Consultant.
2. Atlas, R.M., and R. Bartha. 1986. Microbial Ecology. The Benjamin/Cummings Publishing Company, Inc., New York, NY.
3. Belser, N.O. and Castro, C.E. 1971. Biodehalogenation-The metabolism of the nematocides cis- and trans-3-chloroallyl alcohol by a bacterium isolated from soil," J. Agric. Food Chem. **19:**23-26.
4. Bewick, D.W. 1986. Stereochemistry of fluazifop-butyl transformations in soil. Pestic. Sci. 17:349-356.
5. Chung, K.Y., D.W. Dickson, and L.T. Ou. 1999. Differential enhanced degradation of cis- and trans-1,3-D in soil with a history of repeated field applications of 1,3-D. J. Environ. Sci. Health B34 (5):749-768.
6. Difco Laboratories. 1953. Difco manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures. 9th ed. Difco Laboratories Inc., Detroit, MI.
7. DowElanco. 1996. 1,3-Dichloropropene: A profile. DowElanco, Indianapolis, IN.
8. Hubaux, A. and G. Vos. 1970. Decision and detection limits for linear calibration curves. Anal. Chem. 42:849-855.
9. Lebbink, G., B. Proper, and A. Nipshagen. 1989. Accelerated degradation of 1,3-di -chloropropene. Acta Horticulturae 255:361-371.
10. Leistra, M., A.E. Groen, S.J.H. Crum, and L.J.T. Van der Pas. 1991. Transformation rate of 1, 3-dichloropropene and 3-chloroallyl alcohol in topsoil and subsoil material of f lower-bulb fields. Pestic. Sci. 31:197-207.
11. Mallette, M.F., C.O. Clagett, A.T. Phillips, and R.L. McCarl. 1979. Introductory Biochemistry. Robert E. Krieger Publishing Co., Hungtington, NY.
12. McCall, P. J. 1987. Hydrolysis of 1,3-dichloropropene in dilute aqueous solution. Pestic. Sci. 19:235-242.
13. Noling, J.W., and J.O. Becker. 1994. The challenge of research and extension to define and implement alternatives to methyl bromide. Suppl. J. Nematol. 26: 573-586.

14. Ott, L. 1977. An introduction to statistical mthods and data analysis. Wadsworth Publishing Co., Belmont, CA.

15. Ou, L.T. 1991. Interactions of microorganisms and soil during fenamiphos degradation. Soil Sci. Soc. Am. J. 55:716-722.

16. Ou, L.T. and Thomas, J.E. 1994. Influence of organic matter and soil surfaces on a bacterial consortium that mineralizes fenamiphos. Soil Sci. Soc. Am. J. **58:** 1148-1153.

17. Ou, L.T., K.Y. Chung, J.E. Thomas, T.A. Obreza, and D.W. Dickson. 1995. Degradation of 1,3-dichloropropene (1,3-D) in soils with different histories of field applications of 1,3-D. J. Nematol. 27:249-257.

18. Racke, K.D., and J.R. Coats. 1990. Enhanced Biodegradation of Pesticides in the Environment. ACS Symp. Ser. 426. American Chemical Society, Washington, D.C.

19. Roberts, T.R. and Stoydin, G. 1976. The degradation of (Z)- and (E)-1,3-dichloropropenes and 1,2-dichloropropenes and 1,2-dichloropropane in soil. Pestic. Sci. **7:**325-335.

20. Van Dijk, H. 1980. Dissipation rates in soil of 1,2-dichloropropane and 1,3- and 2,3-dichloropropenes. Pestic. Sci. 11:625-632.

21. Van Waarde, J.J., R. Kok, and D.B. Janssen. 1993. Degradation of 2-chloroallyl alcohol by a *Pseudomonas* sp. Appl. Environ. Microbiol. 59:528-535.

22. Verhagen, C., E. Smit, D.B. Janssen, and J.D. Van Elsas. 1995. Bacterial dichloropropene degradation in soil: Screening of soils and involvement of plasmids carrying the dhlA gene. Soil Biol. Bichem. 27:1547-1557.

23. Yang, R.S.H. 1986. 1,3-Dichloropropene. Residue Reviews 97:19-35.

Table 1. Chemical and physical properties of cis- and trans-1,3-D.

Property	Cis-1,3-D	Trans-1,3-D
Chemical formula	$CHCl=CHCH_2Cl$	
Molecular weight	110.97	
Physical state	Liquid	
Vapour pressure (mm Hg at 25°C)	43	34
Color	Colorless or straw-colored	
Odor	Sweet, penetrating	
Density(at 20°C)	1.205	1.219
Boiling point(°C)	104.1°C	112.6°C
Melting point(°C)	<-50°C	<-50°C
Water solubility(㎍/mL)	2180	2320
Partition coefficient(Log Kow)	2.09	2.04
Henry's law constant (atm/gmol)	$1.8x10^{-3}$	$1.05x10^{-3}$

(DowElanco, 1996; Yang, 1986).

Table 2. Various test media containing various second carbon substrates were used to screen for a second carbon substrate that had the capacity to stimulate the degradation of cis- and trans-1,3-D by the mixed culture.

Medium
Basal mineral-trace mineral media (BMTMM) + enhanced soil (0.01 g/mL)
BMTMM + autoclaved enhanced soil (0.01 g/mL)
BMTMM + soil leachate (0.04 mL/mL)
BMTMM + glucose (200 ㎍/mL)
BMTMM + yeast extract (200 ㎍/mL)
L-broth
BMTMM + L-broth (5 ㎕/mL)
BMTMM + tryptone (200 ㎍/mL)
BMTMM + tryptophan (200 ㎍/mL)
BMTMM + Indole (200 ㎍/mL)
BMTMM + Alanine (200 ㎍/mL)

Table 3. Degradation of cis- and trans-1,3-D in basalmineral-trace mineral medium supplemented with nonsterileenhanced soil (10 mg/mL) or autoclaved enhanced soil(10 mg/mL).

Medium	Degradation
Basal mineral-trace mineral medium (BMTMM) + nonsterile enhanced soil	+
BMTMM + autoclaved enhanced soil	-
BMTMM + autoclaved enhanced soil + nonsterile enhanced soil[a]	+

[a]Nonsterile enhanced soil (10 mg/mL) was added 7 days after autoclaved soil was added

Table 4. Screening for second carbon substrates that stimulated degradation of cis- and trans-1,3-D by the mixed culture grown in basal mineral-trace mineral medium (BMTMM)[a].

Second carbon substrate	Degradation
Soil leachate (40 $\mu\ell$/mL)	+
Soil extract	-
glucose (200 μg/mL)	-
L-broth (Full strength)	-
L-broth (10 $\mu\ell$/mL)	+
Yeast extract (200 $\mu\ell\mu$g/mL)	-
Tryptone (200 μg/mL)	+
Tryptophan (200 μg/mL)	+
Indole (200 μg/mL)	-
Alanine (200 μg/mL)	+

[a]With the exception of soil extract and full strength L-broth, the mixed culture was directly inoculated into the two media without using BMTMM.

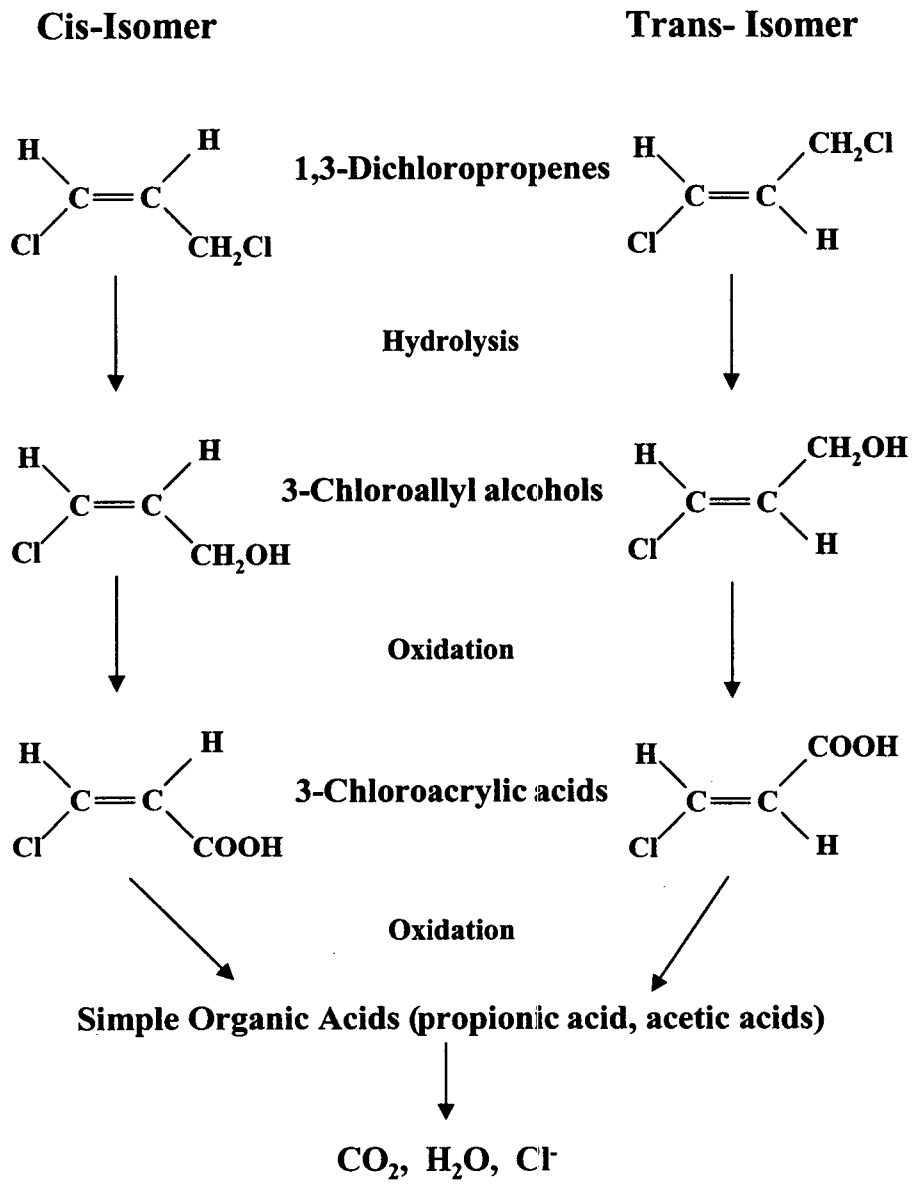

Fig.1. Proposed degradation pathways of cis- and trans-1,3-D in soils (Roberts and Stoydin, 1976).

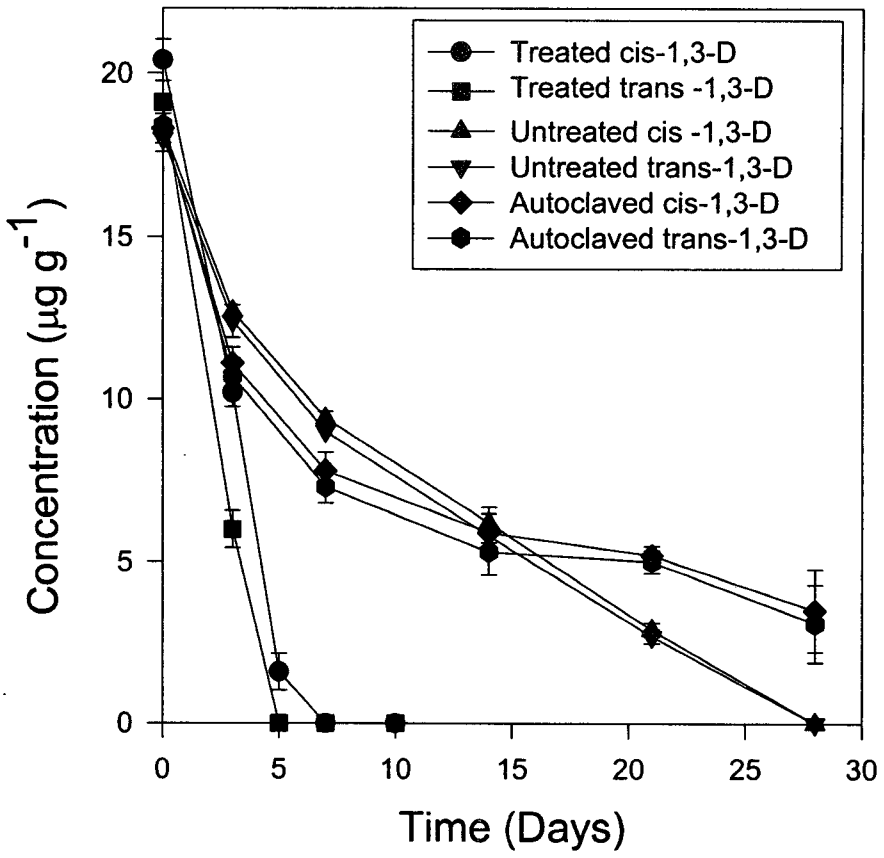

Fig. 2. Degradation of cis- and trans-1,3-D in treated, untreated (control) and autoclaved surface soils.